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BACTERIOLOGICAL INDICATORS OF POLLUTION AND SANITARY STATES OF DŁUGIE WIGIERSKIE LAKE WATER IN THE PRESENCE OF CORMORANTS (*PHALOCROCORAX CARBO*)

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BAKTERIOLOGICZNE WSKAŹNIKI STOPNIA ZANIECZYSZCZENIA I STANU SANITARNO-BAKTERIOLOGICZNEGO WODY JEZIORA DŁUGIEGO WIGIERSKIEGO W WARUNKACH BYTOWANIA KORMORANA CZARNEGO (*PHALOCROCORAX CARBO*)

Praca obejmuje wyniki trzyletnich badań liczebności bakterii wskaźnikowych stopnia zanieczyszczenia (ogólna liczba bakterii oznaczana na agarze bulionowym w temperaturze 22 i 37°C) i stanu sanitarnego (liczba bakterii grupy coli – TC, bakterii grupy coli typu kałowego – FC, paciorkowców kałowych – FS i beztlenowych bakterii przetrwalnikujących redukujących siarczyny – *Clostridium perfringens*) w wodzie Jeziora Długiego Wigierskiego w warunkach bytowania kormorana czarnego (*Phalocrocorax carbo*). Badania przeprowadzono w latach 1998–2001 w 530 próbach wody pobieranych na 14 stanowiskach wyznaczonych w charakterystycznych miejscach jeziora, ze szczególnym uwzględnieniem miejsc lokalizacji siedlisk kormoranów. Większość (powyżej 60%) przebadanych prób wody Jeziora Długiego Wigierskiego mieściła się w I klasie wód o bardzo dobrej jakości. Około 20–30% prób wody mieściła się w II klasie wód dobrej jakości, tylko niewielki procent prób wody wykazywało nieznacznie większy stopień zanieczyszczenia i zaklasyfikowano je do III klasy czystości, tzn. wód o zadowalającej jakości. Wyższym stopniem zanieczyszczenia charakteryzowały się próby wody pobierane zazwyczaj w pobliżu dopływu i odpływu rzeki Dłużanki jak też w sąsiedztwie bytowania kormoranów – w pobliżu wyspy Ostrówek i w strefie przybrzeżnej. Próby wód bardziej zanieczyszczonych bakteriologicznie pobierano częściej w okresie wiosenno-letnim, kiedy aktywność ptactwa wodnego i innych zwierząt w rejonie jeziora była większa. Wskazuje na to niski stosunek FC : FS w większości pobieranych w tym czasie prób wody z jeziora. Niski stosunek liczbowy kałowych bakterii grupy coli do paciorkowców kałowych w większości badanych prób wody sugeruje przeważający udział ptactwa wodnego w zanieczyszczaniu tego zbiornika. Liczebności badanych grup drobnoustrojów najczęściej były pozytywnie skorelowane z wartościami typowych parametrów chemicznych odzwierciedlających zanieczyszczenie wód powierzchniowych: $N\text{-}NH_4$, $N\text{-}NO_2$ i $P_{całk.}$.

Summary

The paper comprises the results of a three-year study on the counts of the bacteria which indicate the degree of contamination (total viable count of bacteria on broth-agar at 22 and 37°C) and sanitary state (total coliforms TC, faecal coliforms FC, faecal streptococci (enterococci) FS and anaerobic sulphite-reducing bacterial spores – *Clostridium perfringens*) in the waters of Długie Wigierskie Lake, inhabited by the black cormorant.

The analyses were carried out in 1998–2001, on 530 water samples collected at 14 sites selected in certain characteristic parts of the lake, taking into account the places were black cormorants dwelled. The results of the analyses were subjected to statistical elaboration by determining the coefficients of the correlation between the counts of each group of bacteria examined and some physico-chemical parameters of the lake's water. Most of the water samples (60%) taken from Długie Wigierskie Lake could be classified as water purity class one, i.e. very good quality, about 20–30% of the water samples as water purity class two (good quality). Only a small percentage of the water samples revealed a slight degree of contamination and those samples were considered to belong to water purity class three, i.e. waters of acceptable purity. More contaminated water samples were typically obtained near the inflow and outflow of the Dłużanka River, and around Ostrówek Island, as well as along the lake shores close to colonies of the black cormorant. Samples of the water which was microbiologically more contaminated were more often collected in the spring and summer, when the activity of waterfowl and other animals in and around the lake was more intensive. This was indicated by a low FC : FS ratio for most of the samples collected in those two seasons of the year. The low ratio of FC : FS in most water samples suggests that the lake water was polluted mainly by the waterfowl. The bacterial counts were most often positively correlated with the values of the chemical indicators which typically show contamination of surface waters: $N\text{-}NH_4$, $N\text{-}NO_2$ and P_{total}.

INTRODUCTION

Until recently, research on environmental pollution focused on urban and industrial sources of contamination [16], but today bacteriological contamination is recognized as an increasing problem, particularly in agricultural lands and wildlife areas [4, 8]. One of the sources of faecal pollution in surface waters are the excrements of farm and wild animals [13], such as manure left on pastures and/or on spread as manure on arable soils, and then washed away into streams, rivers and lakes during heavy rains [22]. Rains and melting snow result in a significant increase in the number of sanitary and other bacteria in surface waters [16, 29, 32]. Ten days after the ice cover disappearance, the counts of water pollution indicating bacteria increase several times [35]. The effect of rain is immediately discernable in surface waters and then, in just a few days, it also becomes evident in groundwater [31]. Incidentally, densities of pollution rate indicating bacteria in watercourses can be significantly different in points distant less than a hundred meters, or during an annual cycle. Marshes and wetlands can serve as a reservoir of bacteriological pollutants, deposited there by animals living in the wild [4, 22]. One possible source of contamination is created by waterfowl, which brings in large amounts of biogenic substances and digestive tract bacteria, including pathogenic ones [19, 28]. Wild living birds can transmit pathogenic bacteria with their faeces which fall on pastures, farm animals' feeds or surface waters [1]. As the populations of wild birds grow, the concentrations of FC in water grow, too. Just after two days of 30 seagulls staying at a lake, the count of those bacteria in the lake water can go up to 200 FC per 100 cm^3 [19]. The influence of birds on the bacteriological state of water depends primarily on the birds' species and number, on the location of their overnight stay, length of stay, time of the day and defecation rate [1]. When discussing the natural conditions present at the Wigry National Park, cormorants are often implied as a possible source of contamination for the lake waters in the park. Large populations of these birds (ca 1000 individuals) spend much of the daytime in trees growing near the shores of Długie Wigierskie Lake. Their faeces and pellets, which gather under the pine trees, can be washed away into the lake. Whether they add to the pollution of the lake, and if yes, to what extent they contaminate the lake's open water are the questions discussed in this paper.

DŁUGIE WIGIERSKIE LAKE

The complex of lakes: Długie, Muliczne and Okrągłe, lies north of the south-western bay of Wigry Lake (Wigierski Bay). These three adjacent, post-glacial lakes had been separated from the lake basin of pre-Wigry Lake by evolution of lakes. The aggregate catchment of the above lakes comprises "Długie Lake" nature reserve, set up in 1985, which contains some forest, marshes and the lakes: Długie, Muliczne and Okrągłe, of the total area of 2.94 km^2. The reserve was established to protect the lakes inhabited by beavers and a large colony of cormorants, and to conserve rich water and peat vegetation cover, valuable forest communities with monumental oak trees. Długie Wigierskie Lake lies at 131.9 m above sea level; the water surface area is 0.8 km^2. The maximum depth of the lake is 14.8 m, averaging at 6.4 m. The lake is elongated, of an irregular shape, with two distinct parts separated from each other with a rather deep narrowing. The maximum length of the lake is 2075 m, and the maximum width reaches 775 m. The total length of the shoreline, which creates smaller and larger bays, is 6.2 km. The holding capacity of the lake is moderate and equals ca 5227.6 thousand cubic meters. The lake basin is varied, with a moderately varied bottom. The littoral zone is relatively narrow, and at places the slopes are steep [15]. Długie Wigierskie Lake has three small islands (Ostrówek, Szaniec and Grądzik) of the total land area of 0.016 km^2. The lake is connected with other water bodies through quite broad canals – to the north a canal links this lake with Muliczne Lake, and to the east – Długie Wigierskie Lake is connected with Okrągłe Lake. The lake and its catchments lie in lowland, young glacial landscape, which represents post-lake sander. The landscape relief is not much varied, hilly, with areas lacking drainage. The catchments of the whole lake canal-connected complex is drained by a watercourse flowing out of Okrągłe Lake, called the Dłużanka or Bystrzyca, which falls into Wigry Lake [15]. Annually the Dłużanka carries away 4.3 million cubic meters of water. The area of Długie Wigierskie Lake's direct catchments is 5.1 km^2 whereas the total area of the whole catchments is 7.5 km^2. The direct catchments comprises pastures and meadows (20% of the total area), arable land (23%) and forested land (57%) [3]. The latter are a northern part of Augustowska Forest, which grows around Wigry Lake. Within the catchments there are a few farmsteads, some summer houses and buildings owned by the Polish National Forests. The lake does not have any registered points of pollution [15]. At present, Długie Wigierskie Lake is inhabited by about 1000 cormorants, which have settled on Ostrówek Island and at two other sites near the lake shore in the north-eastern part of the lake. This is not a nesting colony as the birds come to the lake only to feed.

MATERIALS AND METHODS

Sample collection

Taking into consideration the location of the lake's deepest points in both parts, and the places where water flows into and out of the lakes, as well as the sites where cormorants stay, 14 sample collection sites were designated on Długie Wigierskie Lake (Tab. 1, Fig. 1). In 1999 water samples were taken from April to October, and between 2000 and 2001 the sampling was carried out from April to November. From 1999 to

2000 water samples were obtained from 14 sites, and in 2001 only 10 sites served for water sampling. At most of the sites water samples were obtained from the depth of 0.3 m under the water surface and 0.3 m above the bottom, but at deeper sites (no 3 and 7) additional samples were taken from the depth of 7 and 5 m, respectively. Surface water was sampled directly to sterile 250 cm^3 bottles with fitted stoppers; samples from deeper levels were taken with a 2 dm^3 Ruttner's device. All water samples were transported from the sampling site to the laboratory in chilling containers at a temperature of 4–6°C. The time that elapsed from the sampling to analysis did not exceed 12 hours. In total, 530 water samples were taken and analyzed. During the experiment the Provincial Inspectorate for Environmental Protection in Suwałki recorded air temperatures, total atmospheric precipitation and power and direction of winds.

Table 1. Location of water sampling sites at the Długie Wigierskie Lake

Location	Site	Number of samples	Depth [m]
Ploso north-west	1 → north part of ploso	46	0.30; 8.20
	2 → south part of ploso	44	0.30; 9.70
	3 → the deepest site	69	0.30; 7.00; 14.50
	4 → narrowness between ploso	46	0.30; 8.20
Ploso south-east	6 → between Szaniec Island and north waterside	30	0.30; 9.20
	7 → the deepest site	69	0.30; 5.00; 9.70
	9 → at east waterside (the presence of cormorants colony site)	45	0.30; 7.70
	10 → near Ostrówek Island (the presence of cormorants colony site)	23	0.30
	11 → between Ostrówek Island and the deepest site	30	0.30; 8.70
	12 → near south waterside (the presence of cormorants colony site)	23	0.30
	13 → near Szaniec Island	29	0.30; 7.70
	14 → between Ostrówek Island and south-east waterside	30	0.30; 7.70
Dłużanka' inflow	5 → inflow of Muliczne Lake	23	0.30
Dłużanka' outflow	8 → outflow of Okrągłe Lake	23	0.30

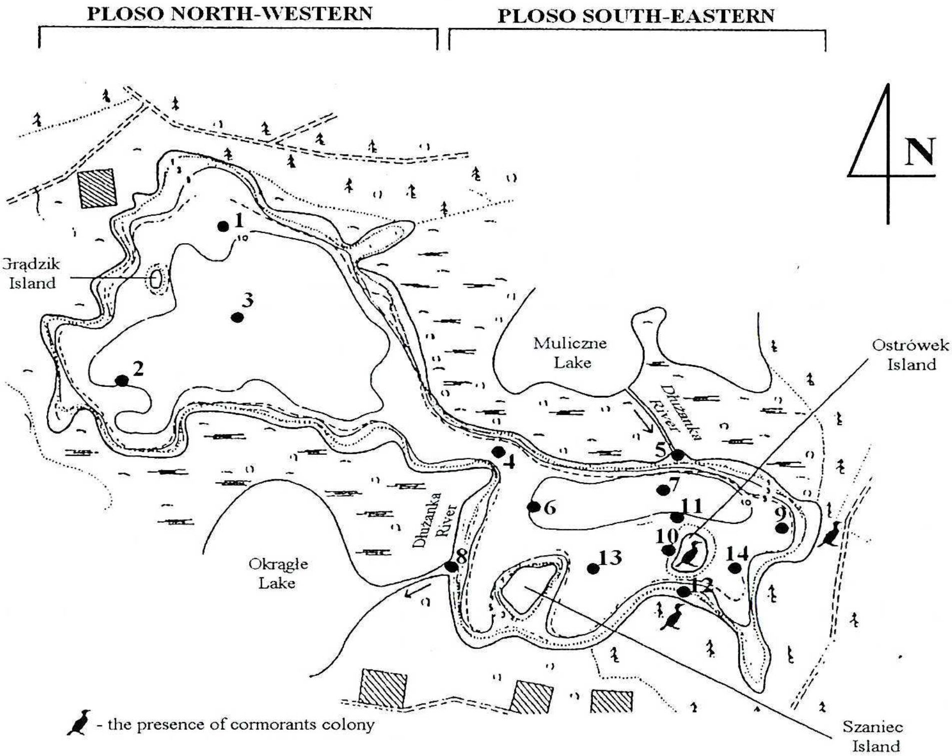

Fig.1. Location sketch of the Długie Wigierskie Lake; 1, 2,... 14 – water sampling sites

Microbiological examination

Bacteriological analyses comprised the following:

1. Total viable counts (CFU/cm^3) of psychrophilic bacteria on broth agar after 72 h incubation at 22°C (TVC 22°C);
2. Total viable counts (CFU/cm^3) of mesophilic bacteria on broth agar after 24 h incubation at 37°C (TVC 37°C);
3. Number (MPN/100 cm^3) of coliforms (TC) on Eijkman medium after 48 h incubation at 37°C;
4. Number (MPN/100 cm^3) of faecal (thermotolerant) coliforms (FC) on Eijkman medium after 24 h incubation at 44.5°C;
5. Number (MPN/100 cm^3) of faecal streptococci (FS) on Slanetz and Bartley medium after 72 h incubation at 37°C;
6. Number (MPN/100 cm^3) of anaerobic spore-forming, sulphite reducing bacteria (*Clostridium perfringens*) on Wilson-Blair' medium after 18 h incubation at 37°C (after 10 minutes pasteurization of water samples at 80°C) [6].

Each measurement was done in three simultaneous repetitions with the same sample of water. TVC 22°C and 37°C were measured according to the guidelines laid down in the relevant Polish Standard. MPN of TC, FC and FS in 100 cm^3 were measured according to the guidelines laid down in the A.P.H.A. Standard Methods [2] and read out from Mc Crady's tables [21].

Simultaneously, the physico-chemical properties of the water in Długie Wigierskie Lake were tested at the chemical laboratory of the Provincial Inspectorate for Environmental Protection in Białystok (subsidiary division in Suwałki), according to the binding Polish Norms. In addition, certain meteorological observations were completed, including the mean daily and monthly temperature during the experiment, the total atmospheric precipitation for 7 days prior to each water sampling, and the power of wind in the 2 days preceding the sampling. Both the physico-chemical and meteorological data were taken into account while performing the statistical evaluation and the detailed results can be obtained from the authors.

Statistical evaluation

The results of the microbiological, chemical and meteorological examinations were subjected to statistical evaluation by determining the correlation (estimation by Spearman' correlation) between a given set of parameters with simple correlation coefficients. In order to obtain information concerning potential differences between bacteria numbers (dependent variables) for various time and place of samples collecting (independent variable), a single factor analysis of variance (ANOVA) was conducted, verifying the hypothesis of the equality of means ($H_0 : x_1 = x_2 = \ldots = x_5$) at the level of significance $\alpha = 0.05$, assuming that the variance for the numerousness of the bacteria groups under study are uniform. The uniformity of variance was tested with Levene's test. If the test proved significant, the hypothesis was rejected. Next, the Kruskal-Wallis' test was applied, which is a non-parametric equivalent of the analysis of variance [33].

RESULTS

Pollution indicating bacteria

The total viable count of bacteria determined on broth-agar at 22°C in the water of Długie Wigierskie Lake varied from 2 CFU/cm^3 at site 7 (at a depth of 5 m) in October 1999 to 16320 CFU/cm^3 at site 4 (at a depth of 0.3 m) in July 2000. On average, smaller counts of bacteria were found in 1999; the counts determined in 2000 and 2001 were higher. In an annual cycle, lower numbers of bacteria appeared in spring than in summer (Tab. 2). The total count of bacteria determined on agar broth at 37°C ranged from 0 CFU recorded sporadically in 1999 and 2000 in water samples from different sampling sites, to 16520 CFU/cm^3 at site 2 (above the bottom) in July 2001. On average, lower bacterial counts were determined in 1999; in the years 2000 and 2001 the numbers of bacteria were less than ten- to less than twenty-fold higher. In an annual cycle, the lowest bacterial counts were found in October 1999 and in April 2000 and 2001; the highest counts of bacteria were discovered in June, July and August 1999 and 2000 as well as in July, August and September 2001 (Tab. 2).

Table 2. Total viable counts at 20 and 37°C in Długie Wigierskie Lake water in 1999–2001 (a – mean; b – ranges)

Site	Number of samples	Total viable counts at:					
		22°C			37°C		
		CFU/cm³					
		1999	2000	2001	1999	2000	2001
1	46	95[a] 31–236[b]	1440 15–10500	690 60–1700	36 0–181	655 5–7490	264 2–1915
2	44	179 33–864	1230 12–10360	2101 83–9040	47 3–276	228 4–790	1536 22–16520
3	69	113 5–295	581 6–3855	822 71–3780	77 3–369	297 1–2520	248 2–1850
4	46	83 12–151	1505 15–16320	927 87–3010	43 1–148	709 11–8660	267 2–1370
5	23	195 78–360	299 15–585	1199 139–2360	108 11–209	166 27–535	2199 13–10960
6	30	62 16–180	1360 29–15800	–	103 0–1100	586 22–7380	–
7	69	98 2–293	632 17–2610	1440 59–7090	42 0–110	330 0–1560	650 3–5800
8	23	133 37–356	423 11–1610	604 121–1500	129 9–300	101 28–285	233 9–1040
9	45	98 17–258	765 5–2500	811 79–2940	44 2–148	203 32–880	611 3–4480
10	23	85 6–189	426 14–1075	941 162–2030	110 2–645	134 30–215	449 7–2250
11	30	102 10–232	848 11–4770	–	41 1–168	445 6–3125	–
12	23	136 71–275	1323 38–4495	2021 285–5940	97 1–222	459 21–1262	1248 18–6820
13	29	104 24–169	1237 17–10240	–	51 2–210	203 16–964	–
14	30	91 21–300	1658 14–15720	–	42 0–161	279 8–980	–

Sanitary state bacteria

The counts of TC and FS in Długie Wigierskie Lake waters varied from < 3 to 1400 MPN/100 cm³ at different sites. The count of faecal coli bacteria varied from < 3 in most of the analyzed samples to 4 MPN/100 cm³ at site 12 in 1999, 1400 at sites 3, 4, 6 and 14 in 2000 and 1100 MPN/100 cm³ at sites 2, 5 and 12 in 2001 (Tab. 3). In an annual cycle lower counts of sanitary state indicating bacteria were noticed in April, May, July and September 1999, in April, June, September and November 2000 and in April, May, October and November 2001; the counts of these bacteria were higher in June, August and September 1999, in July and August 2000 and in May, July and September 2001. Anaerobic sulphite-reducing bacterial spores (*Clostridium perfringens*) were determined only in the near-bottom water in April 2000 at sites 3, 8, 10, 11, 13 and 14. The count of these bacteria was then 40 MPN/100 cm^3 (Tab. 4). The ratio of faecal coliforms to faecal streptococci in most analyzed water samples was less than 0.043. This ratio went above 4 only in the water samples taken in April, May and July 2000 and in August 2001 (Tab. 5).

Table 3. Number of TC – total coliforms and FC – faecal coliforms in Długie Wigierskie Lake water in 1999–2001 (a – mean; b – ranges)

Site	Number of samples	Number of coliform bacteria			Number of faecal coliform bacteria		
		MPN/100 cm^3					
		1999	2000	2001	1999	2000	2001
1	46	25[a] < 3–95[b]	98 4–450	46 15–150	– < 3	8 < 3–45	6 < 3–25
2	44	33 4–150	164 4–1100	520 9–1400	– < 3	11 < 3–95	108 < 3–1100
3	69	80 < 3–1100	121 4–1400	131 4–1400	– < 3	64 < 3–1400	24 < 3–450
4	46	55 4–450	180 9–1100	154 14–1400	– < 3	99 < 3–1400	26 < 3–300
5	23	589 4–1400	264 9–1100	629 40–1400	– < 3	22 < 2–150	165 < 3–1100
6	30	29 < 3–95	137 9–1400	–	– < 3	93 < 3–1400	–
7	69	95 < 3–1100	226 4–1400	307 9–1400	– < 3	7 < 3–45	14 < 3–150
8	23	28 4–45	359 15–1100	206 15–1100	– < 3	29 < 3–200	7 < 3–25
9	45	24 < 3–95	104 20–450	192 9–1100	– < 3	14 < 3–150	7 < 3–25
10	23	32 4–95	89 25–150	402 75–1400	– < 3	5 < 3–25	14 < 3–45
11	30	13 < 3–45	184 4–1400	–	– < 3	33 < 3–450	–
12	23	288 7–1400	521 25–1100	646 45–1400	1 < 3–4	206 < 3–1100	193 < 3–1100
13	29	31 < 3–150	216 4–1400	–	– < 3	98 < 3–1100	–
14	30	48 < 3–450	209 9–1400	–	– < 3	94 < 3–1400	–

Table 4. Number of FS – faecal streptococci and anaerobic spore-forming, sulphite reducing *Clostridium perfringens* in Długie Wigierskie Lake water in 1999–2001 (a – mean; b – ranges)

Site	Number of samples	Number of faecal streptococci			Number of anaerobic spore-forming, sulphite reducing bacteria		
		MPN/100 cm³					
		1999	2000	2001	1999	2000	2001
1	46	15[a] 3–30[b]	59 < 3–450	207 < 3–1400	– < 3	– < 3	– < 3
2	44	29 < 3–160	68 < 3–450	248 4–1400	– < 3	– < 3	– < 3
3	69	48 < 3–300	139 < 3–1400	87 < 3–450	– < 3	5 < 3–40	– < 3
4	46	46 4–450	40 9–95	205 9–1400	– < 3	– < 3	– < 3
5	23	111 < 3–250	310 9–1100	338 9–1400	– < 3	– < 3	– < 3
6	30	17 < 3–95	129 < 3–1100	–	– < 3	– < 3	– < 3
7	69	83 < 3–1100	142 < 3–1400	99 25–450	– < 3	– < 3	– < 3
8	23	25 9–95	244 9–1400	232 9–1100	– < 3	5 < 3–40	– < 3
9	45	16 < 3–75	48 4–200	47 9–150	– < 3	– < 3	– < 3
10	23	28 4–75	35 4–95	99 9–250	– < 3	5 < 3–40	– < 3
11	30	14 < 3–65	112 < 3–450	–	– < 3	5 < 3–40	– < 3
12	23	67 9–250	540 3–1400	384 25–1100	– < 3	– < 3	– < 3
13	29	14 < 3–40	220 9–1100	–	– < 3	5 < 3–40	– < 3
14	30	11 < 3–30	215 < 3–1400	–	– < 3	5 < 3–40	– < 3

Table 5. Percentage distribution of the values of ratio FC : FS in Długie Wigierskie Lake water during whole time of study

FC:FS ratio	1999 year							2000 year								2001 year							
	months																						
	IV	V	VI	VII	VIII	IX	X	IV	V	VI	VII	VIII	IX	X	XI	IV	V	VI	VII	VIII	IX	X	XI
> 4	0	0	0	0	0	0	0	12	4	0	12	0	0	0	0	0	0	0	0	22	0	0	0
4.0–0.7	0	0	0	0	0	0	0	4	4	8	46	12	0	0	4	0	17	0	17	11	11	0	6
< 0.7	0	0	0	0	0	0	0	19	34	8	42	38	27	12	8	6	44	11	17	61	39	11	22
< 0.043	100	100	100	100	100	100	100	65	58	84	0	50	73	88	88	94	39	89	66	6	50	89	72

Table 6. Statistic estimation by coefficient of simple correlation between numbers (CFU/cm^3) of studied microorganisms (TVC 22 and 37°C – total viable count of psychrophilic and mesophilic bacteria, TC – number of coliforms, FC – number of faecal coliforms, FS – number of faecal enterococci) and some chemical compounds in Długie Wigierskie Lake water; important correlations ($p < 0.05000$) marked (n – number of samples; r – the crucial value of correlation coefficient)

Variable	$N\text{-}NO_3$ [mg/dm^3]	$N\text{-}NO_2$ [mg/dm^3]	$N\text{-}NH_4$ [mg/dm^3]	N_{org} [mg/dm^3]	$P_{tot.}$ [mg/dm^3]	$P\text{-}PO_4$ [mg/dm^3]	Chlorophyll a [μg/dm^3]	Seston [mg/dm^3]	Secchi' disck [m]	Oxygen [mg/dm^3]	Temp. of water [°C]	Rainfall [mm]	Amount of month' rainfall
n = 176; r = 0.148													
TVC 22°C	-0,354	0,169	0,197	-0,003	0,151	-0,049	-0,322	-0,013	-0,114	-0,284	0,103	0,011	0,202
TVC 37°C	-0,209	0,039	0,095	-0,247	0,203	0,094	-0,236	0,227	-0,407	-0,203	0,316	0,144	0,251
TC	-0,156	-0,103	0,004	-0,013	0,010	-0,072	-0,032	-0,041	-0,247	-0,013	0,197	-0,098	0,026
FC	-0,206	0,096	0,142	-0,139	0,067	-0,053	-0,159	-0,035	-0,314	-0,186	0,096	0,108	0,306
FS	-0,344	0,048	0,152	0,037	0,013	-0,088	-0,136	-0,159	-0,096	-0,153	0,127	0,077	0,020

Table 7. Bacteriological evaluation of water quality of the Długie Wigierskie Lake using criteria in Regulation of the Ministry of Environment from 11th February 2004 [13]; percent of water samples in the class ([1] – FC – number of coliforms; [2] – TC – number of faecal coliforms; [3] – number of samples in the bracket)

Bacteriological water quality criteria		Class of water quality	Sites			
	Number of bacteria		Ploso north-west (1, 2, 3, 4)	Dłużanka' inflow (5)	Ploso south-east (6, 7, 9, 10, 11, 12, 13, 14)	Dłużanka' outflow (8)
FC [1] MPN/100 cm^3	20	1	87.3	87.0	86.7	78.3
	200	2	10.2	13.0	10.4	17.4
	2.000	3	2.5	0.0	2.9	4.3
	20.000	4	0.0	0.0	0.0	0.0
	> 20.000	5	0.0	0.0	0.0	0.0
			(205)[3]	(23)	(280)	(23)
TC [2] MPN/100 cm^3	50	1	68.7	61.3	61.1	30.4
	500	2	25.9	21.7	30.4	39.2
	5.000	3	5.4	13.0	8.6	30.4
	50.000	4	0.0	0.0	0.0	0.0
	> 50.000	5	0.0	0.0	0.0	0.0
			(205)[3]	(23)	(280)	(23)

Statistical evaluation of the research results

Significant positive correlation was determined between:

1. water temperature and total viable count of bacteria determined on broth-agar at 37°C and the number of TC;
2. total atmospheric precipitation and total count of bacteria determined on nutrient agar at 22 and 37°C and the count of FC;
3. P_{total} content and total viable count of bacteria determined on broth-agar at 22 and 37°C;
4. $N\text{-}NO_2$ and $N\text{-}NH_4$ concentrations and total count of bacteria determined on broth-agar at 22°C (Tab. 6).

No statistically significant differences were found between numbers of the analyzed groups of bacteria in water samples from each site except TC and FC (detailed results can be obtained from the authors). The lowest mean counts of these bacteria were determined at site 8, at the outflow of the Dłużanka River, and at site 12 near the place where cormorants nested.

The number of indicatory bacteria counts and degree of water pollution of Długie Wigierskie Lake

When assessing the bacteriological quality of the water in Długie Wigierskie Lake according to the requirements established in the Regulation of the Ministry of Environment of 11th February 2004 on the classification of surface waters [30], we found out that most (over 60%) of the analyzed water samples taken from Długie Wigierskie Lake was within class I of very good quality waters. About 20 to 30% of those water samples belonged to class II (good quality), and only a few percent of the samples revealed some signs of contamination, thus they were classified as class III of water purity, i.e. satisfactory water quality. The water sampled from site 5 (located at the inflow of the Dłużanka River), 8 (near the outflow of the Dłużanka) and from the sites in the eastern part of the lake, close to where cormorants lived, typically showed the most inferior water quality. During the time of the experiment, the highest percentage of such samples was recorded in 2001; in an annual cycle such samples were most frequent in summer (Tab. 7).

DISCUSSION

Although the numbers of pollution indicating bacteria (total count of bacteria determined on nutrient agar at 22 and 37°C) and sanitary state indicating bacteria (counts of TC, FC and FS) in the water samples of Długie Wigierskie Lake, analyzed in 1999–2001, were most often higher than those recorded in the summer of 1995 [25], the vast majority of the samples (90%) contained such low numbers of the bacteria that enabled us to classify the waters as very good or good quality. More severe bacteriological contamination discovered at sites 5, 8 (the inflow and outflow of the Dłużanka River) and 12 (close to colonies of cormorants) could have been associated with the washing out of the birds' faeces deposited within their colonies. The available literature does not provide the data on the qualitative and quantitative composition of bacteria of cormorants' faeces. This bird species feeds on fish, and its feeding area reaches 30 km [20]. The colony of cormorants at Długie Wigierskie Lake is a non-nesting assemblage and the birds mainly catch fish on the adjacent lakes, and less often on the lake we examined. The influence of the cormorants on the bacteriological cleanliness of the lake may depend directly on the microflora of the fish they eat, and indirectly it could be linked to the sanitary and bacteriological state of the water bodies where those cormorants catch fish. Most of those water reservoirs lie within Wigry National Park, and are characterized by a nearly negligible level of contamination [17, 25]. There are some exceptions, however, such as heavily polluted areas of Wigry Lake near the Hańczańska Bay and Wigierki Bay, and the water of the Czarna Hańcza River from below the sewage and wastewater treatment plant in Suwałki to the river mouth near the Hańczańska Bay [18, 23, 26]. Heavier water bacteriological contamination of the waters of Długie Wigierskie Lake at site 5, near the water inflow from Muliczne Lake, could be connected with the disruption of the surface layer of the bottom deposits in the Dłużanka, and with the release of large amounts of bacteria to water, later transported to Długie Wigierskie Lake. The references say [7, 24, 26, 27] river or lake bottom deposits contain much more bacteria than the water above. The surface layer of the bottom deposits in the Dłużanka may be caused at any time of year by wild animals inhabiting the territory near the lakes, and looking for watering places in winter. The Dłużanka is a relatively long channel with a shallow and muddy bottom. Its catchments, covering an area of 1.6 km^2, are overgrown with pine forest with some spruce and birch trees [15]. In summer, when the water supply of Muliczne Lake from two springs in the lake's bottom balances intense water evaporation, the water flow in the channel is nearly unnoticeable. However, in colder seasons of the year this water flow becomes so intense that it prevents the river water from freezing even at very low freezing temperatures. The surface layer of the bottom deposits' disturbance in the Dłużanka River can also occur during heavy rains in spring and summer, or in spring when snow is melting; in short, when the water flow in the river increases. The growth in the count of sanitary state indicating bacteria in water following rain, caused by accumulation of the surface outflow and transfer of contaminants from the surface layer of bottom sediments has been observed by several authors, including Crabill *et al.* [17]. The ratio of FC to FS being lower than 0.043 in ca 75% of the water samples (taken from Długie Wigierskie Lake) suggests the lake was predominantly polluted by wild animals. This ratio rose above 4.0 only in April, May and July 2000 and in August 2001, and implied human source of pollution, which could be indicative of some uncontrolled discharged of municipal sewage. On the other hand, the presence of numerous waterfowl on and around Długie Wigierskie Lake might undermine the conclusion that the lake water contamination was caused by man. This ratio being higher than 4.0 is typical of some bird species, for example gulls, tundra swans, Canada geese and mallards [11, 14]. The sporadic occurrence of anaerobic sulphite reducing bacterial spores (*Clostridium perfringens*) in the water samples taken from Długie Wigierskie Lake at sites 3, 8, 10, 11, 13 and 14 in 2000 (up to 40 MPN/100 cm^3) seems to suggest, however, that the water may have been contaminated by people and/or animals, possibly in some more distant past (in the absence of FC). Seasonal variability in the counts of water pollution indicating bacteria (total viable count of bacteria determined on broth-agar at 22 and 37°C) in Długie Wigierskie Lake waters could have been due to the fluctuation in the quality and amounts of organic and mineral compounds, both autho- and allochtonous in origin. The counts of bacteria gradually increasing from spring to summer could be associated with the melting of the ice cover on the lake, the inflow of melted snow from the lake's catchments and transport of soil and intestine bacteria to the lake during storms. Seasonal blooms and subsequent death of algae can readily provide water with organic matter easily available to bacteria. The periodic decrease in the counts of bacteria during the vegetative season can be attributable to the release of toxic substances by some blue-green and green algae [5]. The maximum counts of water pollution and sanitary state indicating bacteria determined periodically in summer might be related to elevated water temperature, rainfall, higher contribution of organic matter and more intense activity of wild animals and waterfowl. The effect of temperature, atmospheric precipitation, seston and P_{total} concentration on counts of some of the indicating bacteria we analyzed is supported by the correlation coefficients. Extra amounts of indicating bacteria brought by the animals and birds living in and around Długie Wigierskie Lake (including cormorants) could be counteracted by solar radiation, which kills bacteria [9], as well as by the foraging of bacteria and by viral lysis [12, 34, 36, 37].

CONCLUSIONS

1. Most (over 60%) of the analyzed water samples taken from Długie Wigierskie Lake belonged to class I of water purity (very good quality). Around 20 to 30% of the water samples was classified as class II of water purity (good quality), and just a small percentage of the water samples showed signs of pollution and consequently was termed as class III, i.e. satisfactory water quality.
2. The periodic increase in the counts of the pollution and sanitary state indicating bacteria in the waters of Długie Wigierskie Lake in late autumn and in summer could be related to the inflow of faeces left by wild animals near this lake during spring thaw or during rains in summer, and to the activity of water birds.

3. The fact that the cormorants drop faeces on Ostrówek Island and along the shores of Długie Wigierskie Lake may play some role in raising the counts of water pollution and sanitary state indicating bacteria recorded in the course of our experiment.
4. The activity of wild animals in the direct basin of the Dłużanka River could be associated with higher amounts of bacteria determined on nutrient agar at 37°C and of TC and FC as well as FS determined in the water samples taken near the river inflow to Długie Wigierskie Lake.
5. The prevailing contribution of waterfowl and wild animals to the contamination of Długie Wigierskie Lake is confirmed by the ratio of FC : FS, which was less than 0.043 in most of the water samples analyzed at all 14 sampling sites.

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